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New data on puffballs (*Agaricomycetes, Basidiomycota*) from the Northeast Region of Brazil

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Abstract — In order to increase the knowledge of puffballs in Brazil, specimens were collected in the State of Pernambuco, an understudied area in the Northeast Region, between June 2008 and April 2009. *Arachnion album*, *Bovista dominicensis*, and *Morganella fuliginea* are recorded for the first time from this region. *Bovista grandipora*, collected on soil among grass, is described as a new species in the *Bovista delicata*-complex. This species is characterized by a thin whitish exoperidium, an olive-brown endoperidium, punctate to verruculose, apedicellate basidiospores (4–5.5 µm diam.), and capillitium of the *Lycoperdon*-type with large pits in the hyphal units. Descriptions and illustrations, including SEM micrographs of basidiospores, are presented for each of the identified species, and keys to the recorded species of *Arachnion*, *Bovista*, and *Morganella* from Brazil are also provided.

Key words — *Lycoperdales*, gasteromycetes, Neotropical mycobiota

Introduction

Puffballs are gasteroid fungi, usually oval to pyriform in shape, having a peridium that encloses a mass of spores. The basidiospores, along with other elements (e.g., capillitial threads), comprise the gleba, and as the puffball matures a large number of basidiospores can be released through an apical opening (ostiole) or as the peridium disintegrates (Pegler et al. 1995). These species are generally saprobic, terricolous and humicolous; however, a few (e.g. *Morganella* spp.) grow on decomposing wood (Bates 2004).

The true puffballs were traditionally members of *Lycoperdales*, within in families such *Arachniaceae*, *Lycoperdaceae*, and *Mesophelliaceae* (Miller & Miller 1988). Recently, molecular studies have altered the taxonomic arrangement for this basidiomycetous group, and these species are now placed within the *Agaricales*, in the family *Agaricaceae* (Kirk et al. 2008).

The Northeast Region of Brazil comprises nine states and covers an area of nearly 1,560,000 square kilometres (IBGE 2009), an area larger than the territories of Spain, France and Germany combined. Despite the large territorial area and wide assortment of vegetation types, from tropical rainforests to savanna-like vegetation, only a few puffball species are recorded from this region: i.e., *Bovista aestivalis* (Bonord.) Demoulin, *B. pila* Berk. & M.A. Curtis, *B. plumbea* Pers., *B. pusilla* (Batsch) Pers., *Calvatia cyathiformis* (Bosc) Morgan, *C. maxima* (Schaeff.) Morgan, *C. rugosa* (Berk. & M.A. Curtis) D.A. Reid (= *C. rubroflava* (Cragin) Lloyd), *C. sculpta* (Harkn.) Lloyd, *Lycogalopsis solmsii* E. Fisch., and *Lycoperdon perlatum* Pers. (Baseia 2005a, b; Trierveiler-Pereira & Baseia 2009).

This study aims to contribute to the knowledge of gasteroid fungi in the Northeast Region of Brazil. Here we report on four new records of puffballs from the region, one of which is described as a new species.

Materials and methods

Field expeditions were carried out from June 2008 to April 2009 in five remnants of the Atlantic rainforest in the State of Pernambuco, Brazil: Parque Estadual Dois Irmãos (08°00'13"S, 34°56'59"W), Reserva Ecológica de Carnijós (08°08'42"S, 35°04'34"W), Refúgio Ecológico Charles Darwin (07°49'S, 34°56'W), Parque Ecológico João de Vasconcelos Sobrinho (08°22'06"S, 36°01'57"W), Mata do Estado (07°37'21"S, 35°30'19"W); and at the Campus of the Federal University of Pernambuco (08°02'55"S, 34°57'08"W), in the city of Recife.

Macroscopic characters were described based on observations of fresh and dried material, according to Miller & Miller (1988). Colors were determined according to Kornerup & Wanscher (1978). Observations of microscopic characters were made under a light microscope on glass slides mounts (in 5% KOH or Lactophenol Cotton Blue) prepared by taking a small portion of glebal or peridial material from dried specimens. Thirty randomly selected basidiospores were measured under the light microscope at 1000× to determine the range in spore dimensions.

Scanning electron microscopy (SEM) studies were conducted at the Centro de Tecnologias do Gás (CTGÁS) in Natal (RN), Brazil. Sections were removed from dried basidiomata and dusted onto specimen holders attached with double-sided carbon adhesive tape and then coated with up to 15 angstroms of gold-palladium on an Ion Sputter Coater to prepare for scanning electron microscopy (SEM).

Relevant literature (Kreisel 1967, Demoulin 1971, Suárez & Wright 1996, Kasuya et al. 2006) was used in the identification of the material examined, and voucher specimens are preserved in URM (Holmgren & Holmgren 1998).

Taxonomy

Arachnion album Schwein., Schriften Naturf. Ges. Leipzig 1: 59 (1822). Figs 1–4

Basidiomata globose to subglobose (Fig.1), 8–27 mm diam., attached at the base with a whitish mycelia rhizomorph (to 7 mm in length) (Fig. 2). Peridium thin, fragile, white when fresh, golden (4C6) after drying, smooth; dehiscence irregular. Gleba grayish green (1D4), composed of peridioles resembling minute sand grains (Fig. 3). Subgleba absent. Basidiospores ovoid to subglobose (Fig. 4), 4–5(–5.5) × 3–4(–4.5) µm, smooth, hyaline to greenish in KOH, slight thick-walled, with a single oil drop; pedicels typically short, up to 1 µm, occasionally with a long (to 27 µm) sterigmal remnant still attached. Capillitium and paracapillitium absent.

Habitat — growing on soil among grass.

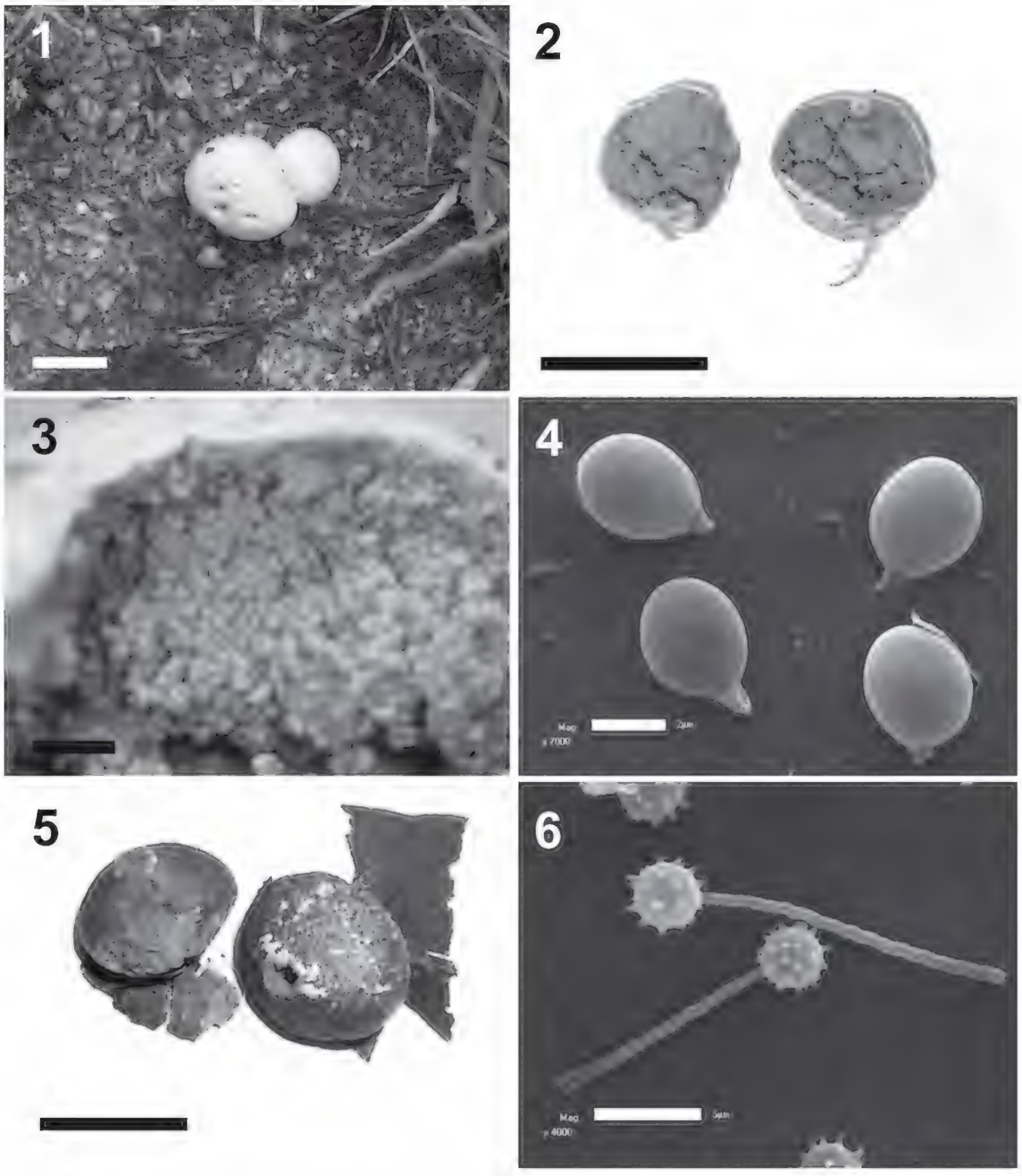
Known distribution — widely distributed throughout the world.

Specimen examined — **BRAZIL. Pernambuco: Recife.** Campus da Universidade Federal de Pernambuco. col. L. Trierveiler-Pereira & V.R. Coimbra. 02.III.2009 (*URM 80075*).

Taxonomic remarks — According to Kasuya et al. (2006), this species resembles *A. drummondii* Berk., *A. iulii* Quadr., and *A. tenerum* (Berk.) Long; however, these can be separated based on basidiospore morphology, coloration of the gleba, and characteristics of the hyphae associated with the peridiole. The description of *A. iriemae* Rick, based on Brazilian material, suggests a puffball that is very close to *A. album*. Rick (1961) separated these species primarily by peridial characteristics (e.g., color, degree of fragility), which can vary as the puffball matures. Although the species may represent a synonym of *A. album*, confirmation of this fact awaits re-examination of the holotype, and it is treated separately here in the key. Four *Arachnion* species are recorded from Brazil and the range of the genus has, thus far, been restricted to the southern parts of the country (Trierveiler-Pereira & Baseia 2009).

Key to the *Arachnion* species recorded from Brazil

1a. Peridium finely warted, pale yellowish when fresh ..... *A. scleroderma*
1b. Peridium smooth, white, grayish or brownish when fresh 2
2a. Peridioles white; peridium brownish, odor fetid ..... *A. foetens*
2b. Peridioles gray to grayish green; peridium white to gray; odor not fetid 3
3a. Peridium grayish, extremely fragile, even in fresh specimens ..... *A. iriemae*
3b. Peridium whitish, gradually becoming more fragile with age ..... *A. album*



FIGURES 1–6. 1–4. *Arachnion album*. 1. Mature basidiomata in situ (scale bar = 2 cm). 2. Mature basidiomata in longitudinal section (scale bar = 2 cm). 3. Peridioles (scale bar = 0.5 mm). 4. SEM of basidiospores (scale bar = 2 µm). 5–6. *Bovista dominicensis*. 5. Mature basidiomata (scale bar = 2 cm). 6. SEM of basidiospores (scale bar = 5 µm).

Bovista dominicensis (Massee) Kreisel, Feddes Repert. 69: 202 (1964). FIGS 5–6,13C

Basidiomata globose, depressed-globose to pyriform, 15–23 mm broad and 12–26 mm high (FIG. 5), base extended into a very small stipitoid part (2–4 mm broad and 3–6 mm high), with ramified whitish rhizomorphs (to 42 mm length). Exoperidium thin, light brown (6D4) to brownish orange (6C3), and

brown (6E7) toward the apex, composed of small, acute granules, appearing somewhat areolate at the apex, but becoming granulose-furfuraceous toward the base. Endoperidium thin, papery, opaque, pale red (7A3) to orange-white (6A2); opening at the apex by a ostiole circular to lobulate, ca. 5 mm diam. Gleba yellowish brown (5E8), powdery to floccose, spore print grayish brown, without an olivaceous tint. Subgleba compact, white, poorly developed. Basidiospores globose, 3.8–4.3 µm diam., verrucose-spinulose, ornamented with scattered hyaline short acute spines or conical warts (Fig. 6, 13C), pale olive-brown in KOH; pedicels long, 10–21 × 1 µm, hyaline, straight and not tapering at the base. Capillitium of the *Lycoperdon*-type; capillitial threads up to 4.5 µm in diam., brownish in KOH, elastic, slightly thick-walled, lacking pits, partly encrusted with a hyaline amorphic substance, dichotomously branched, finely extended at the tips, frequently breaking irregular or rectangular; septa rare, true or false when occurring. Paracapillitium not observed.

Habitat — growing on decomposing leaves and woody debris.

Known distribution — Neotropical.

Specimen examined — **BRAZIL. Pernambuco: Igarassu**. Refúgio Ecológico Charles Darwin. col. J. Pereira. 17.VII.2008 (*URM 80076*).

Taxonomic remarks — This species is morphologically similar to *B. trachyspora* (Lloyd) Kreisel and *B. longissima* Kreisel as these have long pedicellate spores, *Lycoperdon*-type capillitium that is lacking pits, and lack a subgleba (Kreisel 1967). The basidiomata of *B. trachyspora* are typically smaller (usually 10 mm diam.), while those of *B. dominicensis* are larger (reaching 25 mm diam.). Microscopically, the basidiospores of *B. trachyspora* have pedicels that are shorter (to 11 µm in length) than those of *B. dominicensis* (to 38 µm in length). Reports of *B. dominicensis* are rare in Brazil, and so far it was only recorded from two states: Rio Grande do Sul and Espírito Santo (Trierveiler-Pereira & Baseia 2009). This is the first record of *B. dominicensis* from the Northeast Region of Brazil.

Bovista grandipora Trierveiler-Pereira, Kreisel & Baseia, **sp. nov.** Figs 7–10, 13G

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Basidiomata globosa vel subglobosa, 9–24 mm lata, basi funiculis mycelialibus albidis praedita, sine subgleba. Exoperidium tenue, candidum, deinde luteolum, areolatum, glabrum, deinde furfuraceo-areolatum, stratum exterius praecipue hyphis flexuosis compositum, cellulis vesiculosis sparse intermixtis. Endoperidium brunnescens, ore irregulari ad 5 mm amplo dehiscens. Gleba olivaceobrunnea, sine pseudocolumella. Sporae globosae, 3.5–4.5 µm diam., punctatae vel verruculosae, in cumulo olivaceobrunneae, apedicellatae, rudimentis pedicellorum hyalinis, 1–6 µm longis. Capillitium modo generis Lycoperdinis ramificatum; hyphae principales 2.0–6.5 µm crassae, olivaceo-luteolum vel brunneolum, fragile, poris numerosis, conspicuis, ellipticis, 0.5–2 µm latis, dichotomum; septis veribus sparsis praeditum. Paracapillitium nullum. Ad terram.

Holotypus — *Brasilia, Pernambuco, Recife; in herbarium URM conservatus est (URM 80036).*

Etymology — (Lat.) *grandis* = large, conspicuous; *porus* = pit (refers to capillitial threads).

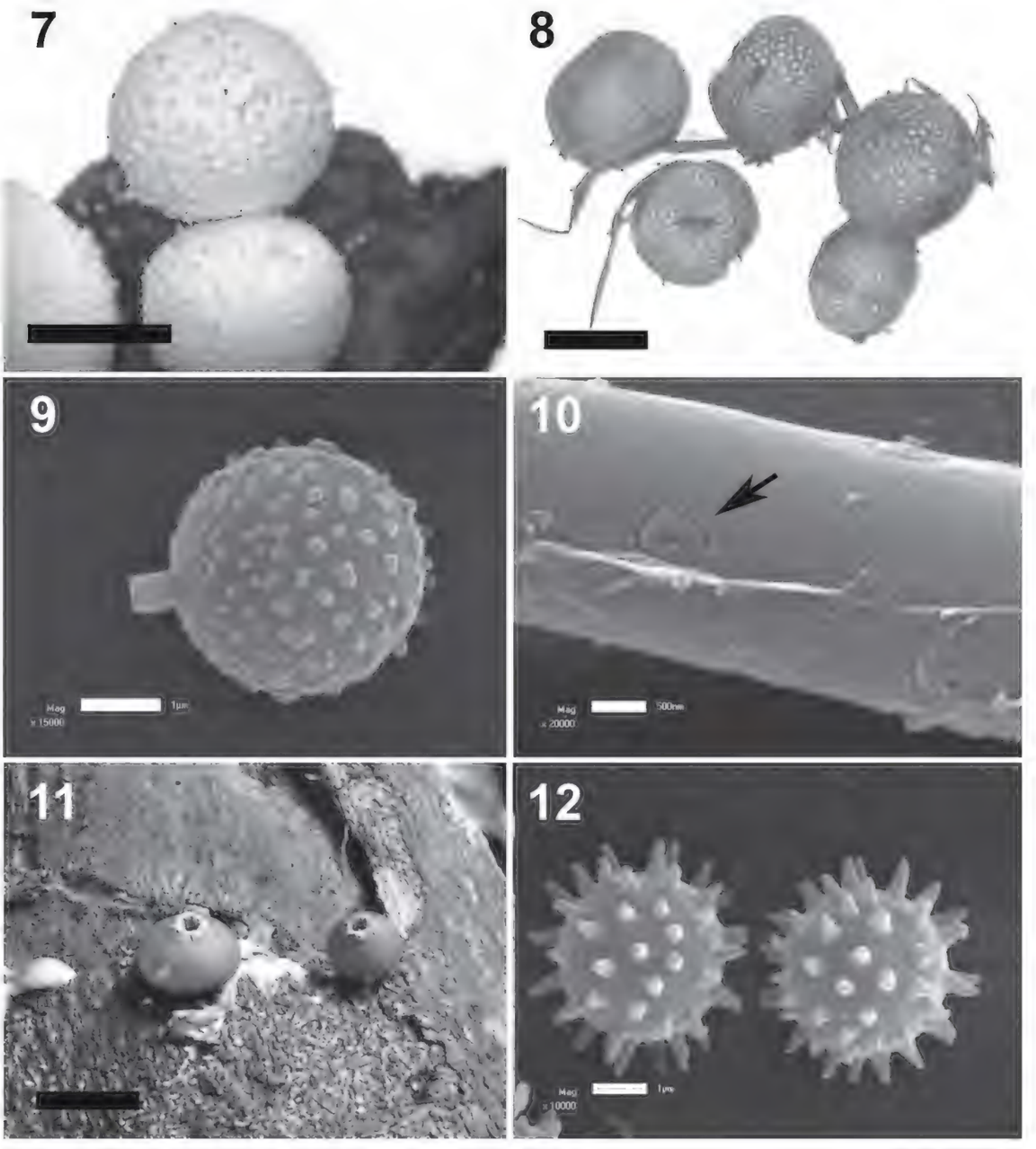
Basidiomata globose, subglobose to depressed globose, 9–24 mm broad and 5.5–21 mm high (Figs 7,8), with whitish to grayish rhizomorphs (to 13 mm length). Exoperidium very thin, white, yellowish white (4A2) to pastel yellow (1A4), smooth to furfuraceous, usually as whitish patches over the endoperidium at maturity, or disappearing completely; exostratum composed primarily of hyaline, septate hyphae and sparsely scattered pseudoparenchymatous cells, mycosclereids absent. Endoperidium thin, papery, light yellow (2A5) to yellow (3B8) when young, later greenish yellow (1B4), olive (3D5), to olive-brown (4D6); ostiole small, irregular in shape, up to 5 mm in diam; Gleba white at first, turning olive-brown (4D5), powdery; pseudocolumella absent. Subgleba absent. Spore print olive green, olive-brown to light brown. Basidiospores globose, 3.5–4.5 mm in diam., punctate to finely verruculose (Figs 9, 13G), pale olive yellow in KOH; pedicels hyaline, short (typically 1 µm length, some reaching 4–6 µm) or completely lacking. Capillitium of the *Lycoperdon*-type; capillitial threads 2–6.5 µm in diam., pale olive-brown in KOH, very fragile, slightly thick-walled, with frequent large (0.5–2.0 µm in diam) elliptical pits (Fig. 10), dichotomously branched; septa true, false septa not observed. Paracapillitium lacking.

Habitat — growing on soil among grass.

Known distribution — Brazil, Puerto Rico, Dominican Republic, Cuba, USA, Spain, Nepal, India, Japan.

Specimens examined — **BRAZIL. Pernambuco: Recife**. Campus da Universidade Federal de Pernambuco. col. V.R. Coimbra & G. Melo. 04.II.2009 (*URM 80067*); same location, col. V.R. Coimbra & G. Melo. 04.II.2009 (*URM 80068*); same location, col. L. Trierveiler-Pereira & V.R. Coimbra. 02.III.2009 (*URM 80069*); same location, col. V.R. Coimbra & F. Wartchow. 13.IV.2009 (*URM 80070*); same location, col. V.R. Coimbra & F. Wartchow. 13.IV.2009 (*URM 80071*); same location, col. L. Trierveiler-Pereira & V.R. Coimbra. 14.IV.2009 (*URM 80072*); same location, col. L. Trierveiler-Pereira & V.R. Coimbra. 14.IV.2009 (*URM 80073*); same location, L. Trierveiler-Pereira & V.R. Coimbra. 14.IV.2009 (*URM 80074*).

Additional material examined by H. Kreisel — **SPAIN. Málaga: Fuengirola**. Castillomoro Sohails. col. J.A. Nannfeldt. 04.X.1957 (*UPS*); **NEPAL**. Dhanhutta, *Pinus roxburghii* forest, col. J.F. Dobremez, 10. 03.IX.1973 (*Herb. Kreisel*); **INDIA**. col S. Ahmad, 13. 1964 and 1965 (*NCU*); **JAPAN. Honshu: Chiba**. Castillomoro Sohails. col. Y. Terashima no. Lp 1, 2, 3, 7, 9. 29.XI.2002 to 03.XII.2002 (*Herb. Kreisel*); **U.S.A. North Caroline**: Chapel Hill. col. J.N. Couch. 22.VI.1922 (*NCU 511*); **Florida: Gainesville**. Alachua Co. col. F.W. Walker. 11.VIII.1924 (*NCU*); **CUBA. La Habana: Calabazar**. col. F. Mazorra. 15.VII.1969 (*HAJB 01178, dupl. in Herb. Kreisel*); **DOMINICAN REPUBLIC. Las Vegas: El Hatillo**. col. C.E. Chardon. 28.VIII.1937 (*NCU 1234a*); **PUERTO RICO. San Juan**, Palominis Id. col. C.E. Chardon. 14.X.1923 (*NCU*).



FIGURES 7–12. 7–10. *Bovista grandipora*. 7. Young basidioma (scale bar = 1 cm). 8. Mature basidiomata (scale bar = 1 cm). 9. SEM of basidiospore (scale bar = 1 μm). 10. SEM of capillitial thread (arrow = capillitial pit; scale bar = 0.5 μm). 11–12. *Morganella fuliginea*. 11. Mature basidiomata in situ (scale bar = 1.5 cm). 12. SEM of basidiospores (scale bar = 1 μm).

TAXONOMIC REMARKS — Macroscopically, *B. grandipora* is typified by basidiomata that grow on soil, have a thin, rather smooth whitish exoperidium (the exostratum being mainly hyphal) and olive-brown endoperidium, and lack a subgleba. Microscopically, the species is characterized by having a capillitium of the *Lycoperdon*-type, fragile capillitial threads with large pits and true septa, and apedicellate, globose, verruculose basidiospores. Although the description above is based on material from Brazil, this species is apparently widely

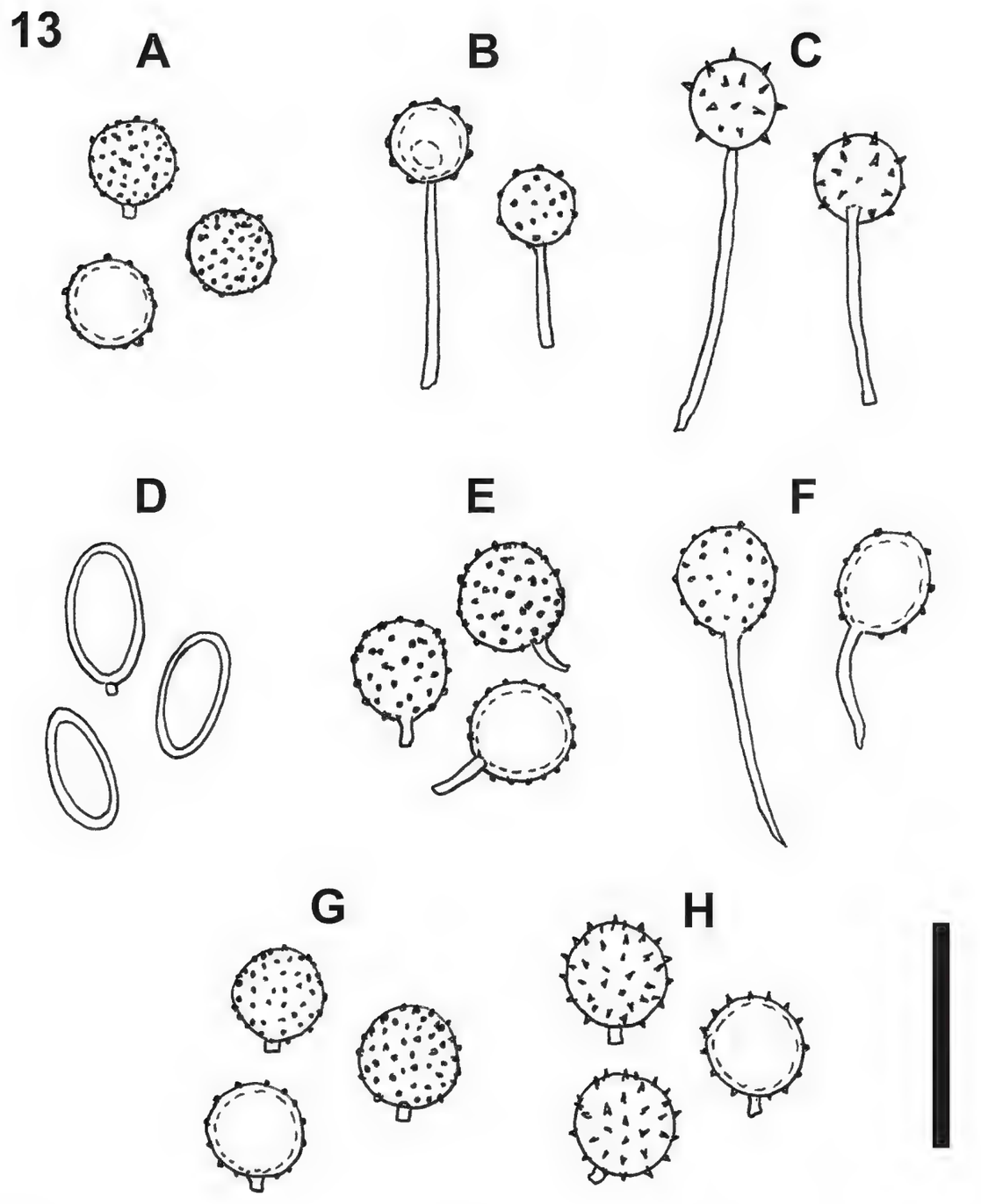


FIGURE 13 (scale bar = 10 µm). Basidiospore of *Bovista* species recorded from Brazil. A. *B. aestivalis*. B. *B. africana*. C. *B. dominicensis*. D. *B. longispora*. E. *B. pila*. F. *B. plumbea*. G. *B. grandipora*. H. *B. pusilla* .

distributed in warmer regions (e.g., tropical, subtropical, and mediterranean to warm-temperate climates) including southern Europe and Asia, southeastern United States, the Caribbean, and South America.

B. grandipora belongs to *Bovista* ser. *Pusillae* Kreisel (Kreisel 1967). It is very close to *B. delicata* Berk. & M.A. Curtis, described from Hong Kong (type in K, PC), which has similar capillitial threads with frequent, large pits; however, it differs from the former by having distinctly pedicellate basidiospores (the pedicels 3–11 in length and somewhat bent). *Bovista pusilla* can be separated from *B. grandipora* as the former has capillitial threads with very small pits, frequent large ellipsoid to claviform cells in the exostratum and a more furfuraceous exoperidium. In a previous publication, Kreisel (1967) included collections of *B. grandipora* within *B. delicata*; however, study of fresh and abundant additional material (see above) indicated that the two taxa should be recognized as distinct species.

Some species in *Calvatia* sect. *Calvatia* (Kreisel 1967) are also characterized by capillitial hyphae with large pits, including the widespread *C. rugosa*; however, the basidiomata of these species are typically much larger. Pits in the capillitial hyphae of *Lycoperdaceae* species range from small to large, slit-like, or may even be lacking. To date, however, the biological function of these pits is not well understood.

Key to the *Bovista* species recorded from Brazil

1a. Capillitium of the *Lycoperdon*- or intermediate type 2

1b. Capillitium of the *Bovista*-type 7

2a. Capillitium of the intermediate type; capillitial threads with small pits ... *B. aestivalis*

2b. Capillitium of the *Lycoperdon*-type; capillitial threads with or without pits 3

3a. Subgleba present 4

3b. Subgleba absent 5

4a. Basidiospores globose, finely punctuate to verruculose, pedicels 4–16.5 µm in length ..... *B. africana*

4b. Basidiospores long-ellipsoid, smooth; pedicels very short or absent .... *B. longispora*

5a. Capillitial threads lacking pits; basidiospores with long pedicels, 10–21 µm in length ..... *B. dominicensis*

5b. Capillitial threads with pits; basidiospores with shorter pedicels, up to 6 µm in length 6

6a. Capillitial threads' pits small, up to 0.5 µm diam. ..... *B. pusilla*

6b. Capillitial threads' pits large, up to 2 µm diam. ..... *B. grandipora*

7a. Exoperidium light brown; basidiospores with short pedicels 1–2 µm in length ..... *B. pila*

7b. Exoperidium grayish brown; basidiospores with longer pedicels, 6–14 µm in length ..... *B. plumbea*

Morganella fuliginea (Berk. & M.A. Curtis) Kreisel & Dring, Feddes Repert. 74: 113 (1967). FIGS 11–12

Basidiomata pyriform, subglobose to depressed-globose, 8–21 mm broad and 6–12 mm high (FIG. 11), with a whitish rhizomorphs attached at the base. Exoperidium thin, at first dull lilac (15C3), grayish lilac (15B2) to lilac (16B3); and white toward the base, becoming reddish brown (8E6), dark brown (7F4) to brown (7E5), and orange (5A6) to pale orange (5A3) toward the base, covered by minute spines when young, becoming velutinous to smooth with maturity, or eventually disappearing; exostratum with spines composed by chains of pseudoparenchymatous cells that are +/– isodiametric, yellowish in KOH, thick-walled, 12–40 × 8–13 µm. Endoperidium thin, flaccid, beige (4C3); ostiole irregular in shape. Gleba grayish green (1C3), powdery. Subgleba pale yellow (1A3), composed of compacted cells. Basidiospores globose, (3.5–)4–5 µm in diam., echinate, slight thick-walled (FIG. 12), yellowish in KOH, with a single oil drop, pedicels absent. Eucapillitium absent. Paracapillitial threads 3–5 µm diam., pale yellow in KOH, thin to slightly thick-walled, lacking pits; septa true.

HABITAT — growing on decomposing wood.

KNOWN DISTRIBUTION — Pantropical.

SPECIMENS EXAMINED — **BRAZIL. PERNAMBUCO: Moreno**. Reserva Ecológica de Carnijós, col. L. Trierveiler-Pereira & J.M. Baltazar, 025. 17.VI.2008 (*URM 80077*); same location, col. L. Trierveiler-Pereira & J.M. Baltazar, 209. 12.III.2009 (*URM 80082*); **Caruaru**. Parque Ecológico João de Vasconcelos Sobrinho, col. L. Trierveiler-Pereira & J.M. Baltazar, 036. 20.VI.2008 (*URM 80078*); same location, col. L. Trierveiler-Pereira et al., 111. 12.VII.2008 (*URM 80080*); **Recife**. Parque Estadual Dois Irmãos, col. L. Trierveiler-Pereira et al., 056. 07.VII.2008 (*URM 80079*); same location, col. L. Trierveiler-Pereira et al., 175. 16.IX.2008 (*URM 80081*); **São Vicente Férrer**. Mata do Estado, col. L. Trierveiler-Pereira & J. M. Baltazar, 210. 19.III.2009 (*URM 80083*); same location, col. L. Trierveiler-Pereira & J. M. Baltazar, 211. 19.III.2009 (*URM 80084*).

TAXONOMIC REMARKS — The species is characterized by strongly echinate basidiospore, and exostratum with chains of pseudoparenchymatous cells. *M. fuliginea* is similar to *M. velutina* (Berk. & M.A. Curtis ex Massee) Kreisel & Dring; however, the latter is distinguished by the presence of setose, thick-walled hyphal elements in the exoperidium (Suárez & Wright 1996). In Brazil, *M. fuliginea* is currently the gasteroid species most widely distributed; being reported from seven states (Trierveiler-Pereira & Baseia 2009). This is the first record of *M. fuliginea* from the Northeast Region of Brazil.

Key to the *Morganella* species recorded from Brazil

1a. Eucapillitium present ..... *M. pyriformis*
1b. Eucapillitium absent 2

2a. Basidiomata growing among dead leaves ..... *M. benjaminii*
2b. Basidiomata growing on rotten wood or humus 3
3a. Exoperidium containing thick-walled, setose hyphal elements ..... *M. velutina*
3b. Exoperidium lacking setose hyphal elements 4
4a. Basidiospores strongly echinate; exoperidium brownish lilac ..... *M. fuliginea*
4b. Basidiospores aspirate; exoperidium white to pale yellow ..... *M. albina*

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